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Metabolism of the Food-borne Carcinogens 2-Amino-3-methylimidazo-[4,5-*f*]quinoline and 2-Amino-3,8-dimethylimidazo[4,5-*f*]-quinoxaline in the Rat As a Model for Human Biomonitoring

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Metabolism of 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,8-dimethylimidazol[4,5-*f*]quinoxaline (MeIQx) and their binding to blood proteins were examined in the rat to develop methods of human biomonitoring. Hemoglobin and serum albumin were among many blood proteins modified. Approximately 0.01% of the dose for both compounds was bound to these proteins, and induction of cytochrome P-450 with polychlorobiphenyls resulted in decreased levels of adduction. Hemoglobin sulfinic acid amide adducts could not be detected for either amine, however, as much as 10% of the IQ bound to albumin was characterized as an N^2-cysteine(34)sulfinyl-IQ linkage. Human dosimetry of these carcinogens through such adducts may prove difficult due to the low levels of protein binding. Major routes of detoxification of both contaminants included cytochrome P-450-mediated ring hydroxylation at the C-5 position followed by conjugation to glucuronic or sulfuric acid. Direct conjugation to the exocyclic amine group through *N*-glucuronidation and sulfamate formation were other important routes of inactivation, but *N*-acetylation was a minor pathway. The *N*-glucuronide conjugate of the mutagenic metabolite *N*-hydroxy-MeIQx was also detected in urine. Rats given MeIQx at 10 μg/kg excreted 20% of the dose in urine within 24 hr and the remainder was recovered in feces. The N^2-glucuronide was the major metabolite found in urine and accounted for 4% of the total dose. The other metabolites cited above also were excreted in urine at amounts ranging from 0.5 to 3% of the dose, whereas 0.5 to 2% was detected as unmetabolized MeIQx. Human hepatic microsomes activated IQ and MeIQx by *N*-hydroxylation, but neither compound was a substrate for hepatic cytosol *N*-acetyltransferases. Both IQ and MeIQx were substrates for hepatic cytosol sulfotransferases, forming the sulfamate derivatives. Immunoaffinity chromatography was used to rapidly purify MeIQx and several metabolites from rat urine as a model for human biomonitoring. Trace levels of MeIQx were detected in urine of humans within 24 hr of consumption of cooked meat by analysis with negative ion GC–MS. Analytical methods are under development for measuring polar metabolites that may be present in human urine.

Introduction

Methods have been developed to rapidly quantitate carcinogenic heterocyclic amines formed in cooked foods such as meat and fish (*1–4*). However, measurement of these contaminants in food only provides a crude estimate of exposure and does not account for absorption and metabolism of these procarcinogens to biologically active species or to detoxified products. Many of these mutagens contain an aminoimidazole group as a common structural feature. 2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) are two important representatives of this class of contaminants. We have used the rat as an animal model to elucidate routes of metabolism and disposition of these two compounds. Particular emphasis has been placed on the analysis of acid-labile sulfinamide protein adducts derived from reactive *N*-hydroxy metabolites and on the analysis of nontoxic metabolites excreted in urine for developing noninvasive methods of human biomonitoring. We have observed that human liver tissue also transforms heterocyclic amines to reactive mutagens as well as

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to detoxified products. Our recent data on the quantification of heterocyclic amines in cooked foods, the use of the rodent model for developing methods of human biomonitoring, and preliminary data from human studies are presented in this article.

Methods

Heterocyclic amines were isolated from heated meat products by adsorption to XAD-2 resin followed by blue cotton treatment or by immunoaffinity chromatogaphy. Quantification was done by HPLC with UV or mass spectrometry detection (*2,3*). Analysis by solid-phase tandem extraction gave similar values, but enabled the measurement of other heterocyclic amines, including 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 2-amino-9H-pyrido[2,3-*b*]indole (2-AαC), which do not cross-react with the antibodies (*4*).

Male Sprague Dawley rats were used for protein adduct and metabolism studies (*5,6*). In brief, hemoglobin was purified by lysis of erythrocytes, followed by gel filtration chromatography. Serum albumin was purified by affinity chromatgraphy using Cibracon Blue conjugated to sepharose. Acid-labile sulfinamide protein adducts were quantitated by HPLC. Metabolites were isolated from biological fluids and reference standards were prepared biosynthetically using rat hepatocyte suspensions or through chemical syntheses (*5,7*).

Analysis of MeIQx excreted in urine of humans following consumption of fried beef was done by the method of Murray et al. (*8*) using negative-ion chemical ionization GC–MS except that an immunoaffinity purification step was included following the acid/base partitioning step (*3*). Three subjects ingested 1 lb equivalent cooked beef prepared under typical household cooking practices and then collected urine for 24 hr. Trideuterio-labeled (N-CD_3)-MeIQx was used as an internal standard. MeIQx and d_3-MeIQx were monitored at the ions m/z 438 and m/z 441 [corresponding to the M-227 ions of the di(3,5-*bis*trifluoromethylbenzyl)derivatives]. Human hepatic microsomes and cytosols were used to measure the apparent rates of *N*-oxidation and *N*-acetylation of heterocyclic amines (*9*). S-9 fortified with adenosine 3′-phosphate-5′-phosphosulfate (PAPS) was used to examine sulfamate formation.

Results

Heterocyclic amines can be detected at the low parts per billion levels in meat and fish prepared under typical household cooking practices. The production of these genotoxins in foods varies greatly and depends on the meat, temperature, and manner of preparation (Table 1).

The major metabolites of IQ and MeIQx that have been identified in urine, bile, and feces of rodents are displayed in Figure 1 (*5,10–12*). All these metabolites are detoxification products with the exception of the *N*-glucuronide conjugate of *N*-hydroxy-MeIQx, which is genotoxic in the presence of β-glucuronidase (*7*).

We examined the metabolism of MeIQx at doses ranging from 0.01 to 20 mg/kg in noninduced rats and in rats pretreated with polychlorinated biphenyls (PCB) (*5*) (Fig. 2). At high dose exposure to MeIQx (20 mg/kg), the sulfamate and N^2-glucuronide were the major metabolites excreted in urine, whereas PCB-pretreated animals excreted greater amounts of conjugates of 5-hydroxy-MeIQx. Cytochrome P-450 induction had no influence on metabolism at the 0.01 mg/kg dose, indicating that under high exposure to MeIQx the cytochrome P-450 is limiting in the noninduced rat and phase II conjugation reactions make a larger contribution to metabolism. Notably, the formation and excretion of the metastable *N*-glucuronide conjugate of *N*-hydroxy-MeIQx was relatively more important at low-dose exposure.

The blood protein binding of IQ was examined at doses from 2 to 150 μmole/kg (*6*). Among many proteins modified, hemoglobin and albumin were modified in a dose-dependent fashion. Albumin bound three to five times more IQ than hemoglobin per mole of protein. The amount of IQ bound to the total albumin pool ranged from 1.4 to 4.3 × 10^{-2}% of the dose (Fig. 3). Analysis of the enzymatically digested peptide fragments revealed many adducts. One adduct was identified as a tripeptide containing an N^2-cysteine sulfinamide-IQ linkage and accounted for as much as 10% of the IQ bound to serum albumin (Fig. 4). A chemically identical adduct was formed *in vitro* when *N*-hydroxy-IQ was incubated with serum albumin. The adduct was labile to acid with quantitative recovery of the parent amine.

Table 1. Heterocyclic aromatic amines in cooked meats and fish.[a]

Meat	3 components of cooking method	Compound, ng/g				
		IQ	MeIQx	4,8-DiMeIQx	PhIP	AαC
Beef steak	Grilled, 190°C, 3 min/side	ND	5.1	1.3	23.5	3.2
	Grilled, 190°C, 7 min/side	ND	8.3	2.0	48.5	8.9
Ground beef	Pan fried, 250°C, 5 min/side	ND	0.7	ND	NA	NA
	Pan fried, 250°C, 10 min/side	ND	1.1	<1	1.2	NA
Ground beef	Pan fried, 270°C, 10 min/side	0.3	4.2	ND	NA	NA
Salmon	Pan fried, 200°C, 3 min/side	ND	1.4	ND	1.7	ND
	Pan fried, 200°C, 9 min/side	ND	4.7	ND	14.0	8.0
Salmon	Barbecued, 270°C, 4 min/side	ND	<1	ND	2.0	2.8
	Barbecued, 270°C, 9 min/side	ND	<1	ND	69.0	73.0
Bacterial-grade beef extract	I	49.0	56.0	ND	NA	NA
	II	70.0	89.0	8.1	NA	NA
Food-grade beef extract	I	6.2	30.5	ND	NA	NA
	II ND	69.0	ND	NA	NA	

Abbreviations: IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; 4,8-DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; AαC, 2-amino-9H-pyrido[2,3-b]indole; ND, not detected (less than 0.3 ppb); NA, not analyzed.

[a]From Turesky et al. (*2,3*), Gross (*4*), and Gross and Grüter (*24*).

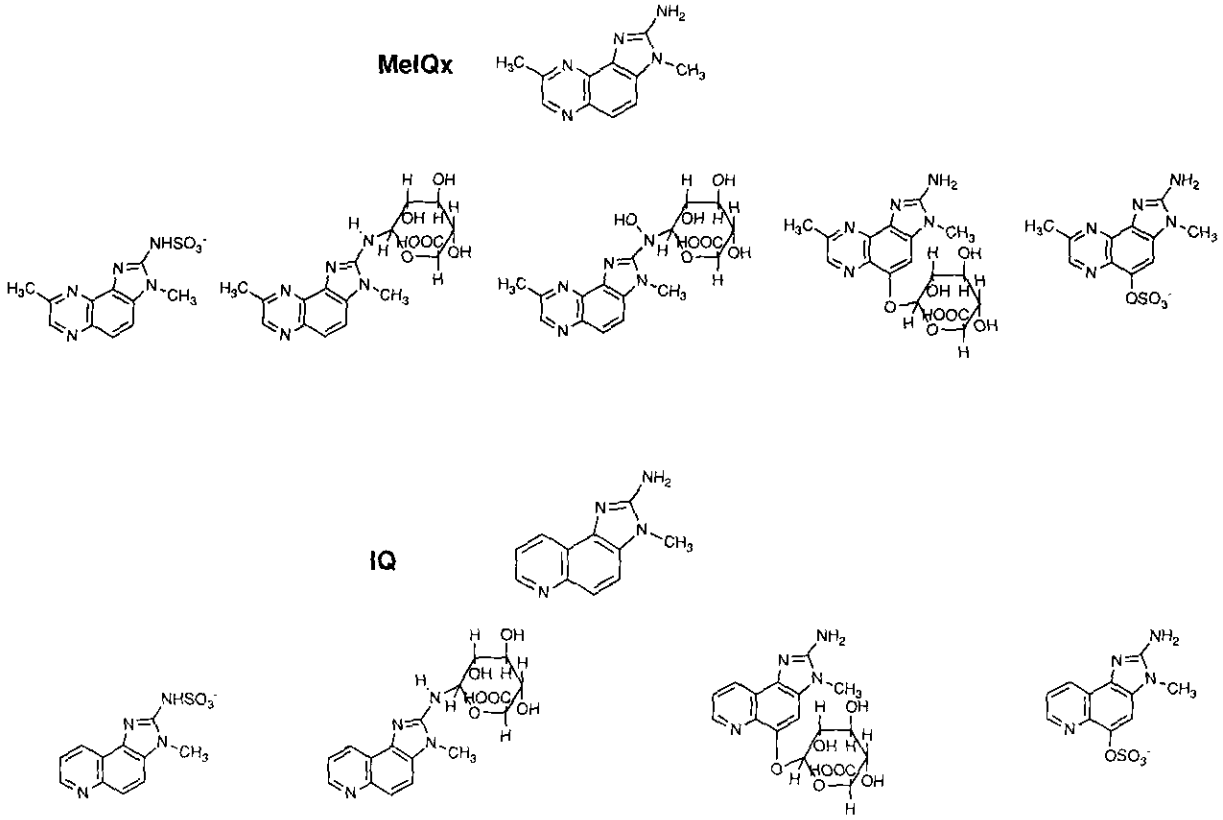

FIGURE 1. Structures of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), and the major metabolites identified in bile, urine, and feces of rodents.

Adducts bound to hemoglobin were briefly examined for the presence of a sulfinamide adduct. Acid hydrolysis released approximately 30% of the bound IQ. Analysis by HPLC revealed that the product(s) were more polar than IQ and excluded the presence of a sulfinamide linkage with hemoglobin. The chemical structures of the polar products have not been elucidated.

The binding of MeIQx to albumin and hemoglobin was several fold lower than that observed for IQ. Hepatic cytochrome P-450 induction with PCB increased the rate of formation of the biologically reactive *N*-hydroxy metabolite by 20-fold in microsomal assays, but resulted in a 10-fold decrease in blood protein binding *in vivo* (*5*). Thus, in contrast to other aromatic amines that are converted to the hydroxylamine and adduct to hemoglobin as a sulfinamide linkage through the 93β cysteine residue *in vivo* (*13*), IQ and MeIQx apparently do not form this adduct at appreciable levels.

The ability of human liver tissue to metabolize heterocyclic amines was examined. The apparent rates of *N*-oxidation were compared with that of 4-aminobiphenyl (ABP), which is regarded as the most potent of arylamine carcinogens. The levels of IQ, MeIQx, and Glu-P-1 *N*-oxidation were about half of that observed for ABP, while that of PhIP was slightly higher (Table 2). Heterocyclic amines were poor substrates for cytosolic *N*-acetyltransferases which was in contrast to the arylamine carcinogens ABP or 2-aminofluorene (*14*), (Table 2). However, human liver S-9 fraction fortified with PAPS was found to readily detoxify IQ and MeIQx through sulfamate formation (R. Turesky, unpublished observations).

The urine of three subjects was examined for MeIQx before and after consumption of 1 lb of cooked beef. MeIQx could not be detected in urine collections before consumption of meat, but the mutagen was detected in all three subjects after the meal (Fig. 5). The amounts of MeIQx recovered ranged in values from 6 to 10 to 18 ng in a 24 hr urine collection. These values are similar to those of Murray et al. (*8*) and indicate that MeIQx is absorbed and extensively biotransformed by humans.

Discussion

Heterocyclic amines formed in cooked foods at the low part per billion level are easily isolated by immunoaffinity chromatography or by solid-phase tandem extraction and then quantitated by HPLC. The class and the amounts of heterocyclic amines produced depend on several parameters including temperature, creatinine content, and meat preparation (*15*). PhIP, followed by AαC and MeIQx, were the most abundant heterocyclic amines found in grilled steak, fried beef, and salmon as well as in barbecued salmon. IQ and MeIQx were the predominant mutagens formed in meat extracts. These amounts are comparable to those reported by other investigators who used far more laborious methods of purification (*15,16*). Based upon these analyses, the daily exposure to each of these amines through the diet may be estimated at approximately 100 ng to 10 μg per day.

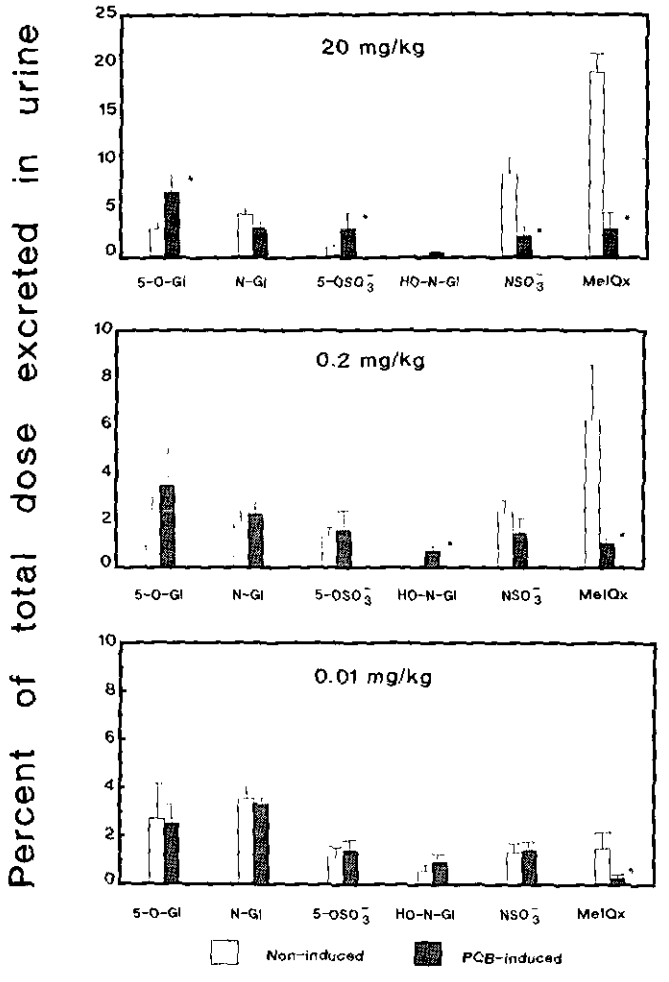

FIGURE 2. Distribution of major urinary metabolites of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) as a function of dose (*5*). (*) $p < 0.05$, Duncan's test, $n = 4$.

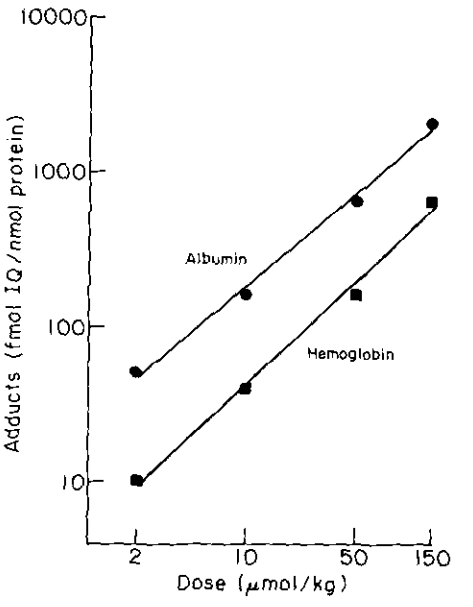

FIGURE 3. *In vivo* formation of albumin and hemoglobin adducts in the rat. The proteins were isolated 20 hr after intragastric administration of [^{3}H(G)]2-amino-3-methylimidazo[4,5-*f*]quinoline. Adduct levels were calculated from the total radioactivity bound to purified proteins (*6*).

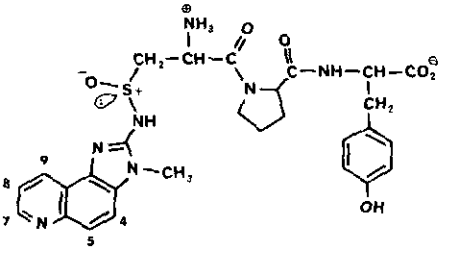

FIGURE 4. Structure of a pair of diastereomeric tripeptides containing an N^2-cysteine(34)sulfinyl-2-amino-3-methylimidazo[4,5-*f*]quinoline linkage, which differ only in the absolute configuration about the sulfur atom.

The metabolism and blood protein binding of IQ and MeIQx in the rodent were examined to develop strategies for human biomonitoring. Measurement of blood protein adducts has been successfully used to assess human exposure and metabolic activation for several different carcinogens, including 4-aminobiphenyl, aflatoxin, and several polycyclic aromatic hydrocarbons (*13,17*). The rodent model has been found to be a good surrogate for blood protein adduct formation by several of these carcinogens including the arylamine ABP, where over 5% of an administered dose is bound to hemoglobin as a sulfinamide linkage. This adduct is the result of a series of reactions between the hemoprotein and the carcinogenic *N*-hydroxy metabolite. The adduct is stable *in vivo*, but it can be cleaved *in vitro* with quantitative regeneration of the parent amine. Thus, measurement of this adduct is an indirect measure of metabolic activation and the biologically effective dose. Relative to ABP, the binding of IQ and MeIQx to hemoglobin was quite low, accounting for approximately 0.01% of the dose, and sulfinamide adducts could not be detected (*5,6*).

Induction of hepatic cytochrome P-450 by PCB, which increased rates of formation of the hydroxylamine metabolites, actually resulted in as much as a 10-fold decrease in protein binding (*5,6*). Incubation of the microsomally generated hydroxylamines or the synthetic *N*-hydroxy derivatives of IQ and MeIQx *in vitro* with erythrocytes generated methemoglobinemia and sulfinamide adduct formation. Thus, the hydroxylamines can penetrate the erythrocyte and react with the hemoprotein. The absence of such an adduct *in vivo* suggests that either very low levels of the *N*-hydroxy metabolites are excreted by the liver into the blood stream or that other routes of biotransformation are of far greater importance than *N*-hydroxylation. Based on the rodent model, formation of sulfinamide adducts in humans would not be expected at appreciable levels, and human dosimetry may prove difficult. In support of this conclusion, a preliminary study assaying for hemoglobin sulfinamide adducts of MeIQx in hu-

Table 2. Comparative rates of *N*-oxidation by human liver microsomes and *N*-acetylation by human liver cytosols.

Substrate	Rate, nmole/min/mg protein ± SD
N-oxidation[a]	
ABP	5.00 ± 0.32
MeIQx	2.98 ± 0.49
IQ	2.30 ± 0.21
PhIP	5.34 ± 0.64
Glu-P-1	2.42 ± 0.14
N-acetylation[b]	
ABP	1.70 ± 0.24
2-AF	2.35 ± 0.55
MeIQx	<0.05
IQ	<0.05
PhIP	<0.05
Glu-P-1	<0.05
PABA	0.11 ± 0.09

Abbreviations: ABP, 4-aminobiphenyl; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; Glu-P-1, 2-amino-6-methyldipyrido[1,2-a:3′, 2′-d]imidazole; 2-AF, 2-aminofluorene; PABA, *p*-aminobenzoic acid.

[a]Rates of *N*-oxidation were determined with hepatic microsomes from the same individual (n = 3,4). Similar results were obtained with microsomes from two other individuals (*9,19*).

[b]These cytosols were selected from rapid acetylator individuals (n = 3) whose status was indicated by the high rates of *N*-acetylation of 2-AF and ABP (*9,14*).

mans showed that the adducts, if present, were below levels of detection using GC–MS (*18*).

Aproximately 0.001–0.004% of an administered dose of IQ bound to serum albumin in the rat as a sulfinamide adduct. In man, with a half-life of 20 days for albumin turnover, the adduct level resulting from chronic exposure is approximately 30 times greater than that produced by a 1-day exposure (*13*). Assuming an human exposure of 1 μg/day with the same adduct binding efficiency as the rat, the amount of serum albumin modfied from chronic exposure in a 70-kg individual containing 130 g of albumin would be approximately 2–8 pg of IQ bound/g of albumin. With this level of modification in humans, methods of enrichment would be required to measure covalent binding of sulfinamide albumin adducts by GC–MS.

We undertook urinary metabolism studies in the rodent with MeIQx at doses from 20 mg/kg to 10 μg/kg, a dose that is only several hundred-fold greater than the daily human exposure. MeIQx was extensively metabolized at the lowest dose examined with only 0.5–2% of the total dose recovered in urine found as unchanged MeIQx. In humans, the amount of MeIQx recovered in urine represented only several percent of the ingested dose and indicates that this procarcinogen is also absorbed and extensively metabolized by humans (*8*). The data of Murray et al. (*8*), as well as data presented here, demonstrate that

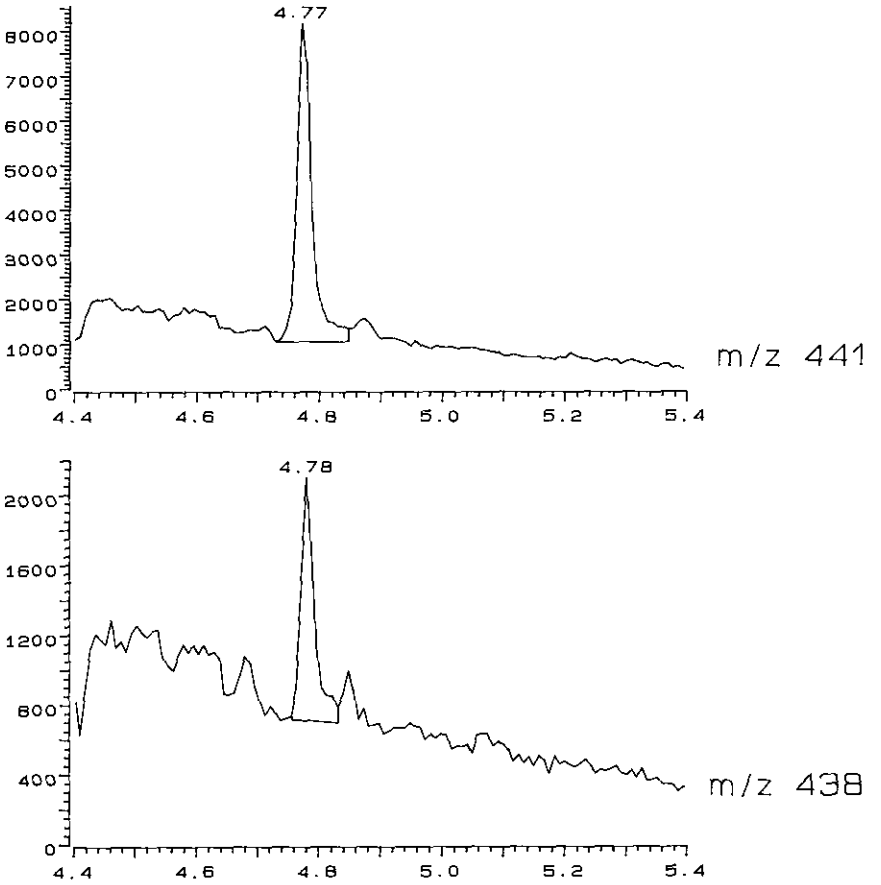

FIGURE 5. Selected monitoring of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) (m/z 438) and its trideuterio-labeled derivative (m/z 441) in human urine following consumption of fried meat. The sample contained 11 pg MeIQx/mL urine, giving a total excretion of 10 ng in the 24-hr urine collection.

GC–MS analysis is sufficiently sensitive to detect MeIQx in urine following consumption of fried meat.

Biotransformation of heterocyclic amines by human tissues resembles that of rodents in several instances. Metabolic activation through *N*-hydroxylation by hepatic cytochrome P-450 IA2 is comparable for both species (*19–21*). Both rodent and human liver *O*-acetyltransferases catalyze binding of the *N*-hydroxy metabolites to DNA-bound products, yet the parent amines are poor substrates for hepatic *N*-acetyltransferases (*9,21*). The *N*-hydroxy metabolites have also been shown to be substrates for human and rodent hepatic glucuronyltransferases (*5,7,10,22*), and the resulting metastable *N*-glucuronide conjugates may be implicated in colorectal carcinogenesis (*22*). A major route of detoxification of heterocyclic amines in rodents, in particular for IQ and MeIQx, is through sulfamate formation. This route of metabolism for aromatic amines is relatively uncommon, owing in part to the labililty of the arylsulfamate bond. We have observed that human liver tissue also converts these two amines to their respective sulfamate derivatives (R. Turesky, unpublished observations).

The major oxidative pathways of detoxification of IQ and MeIQx found in the rodent (*5,10–12*), have also been identified in the monkey (*23*; R.Turesky and E. Snyderwine, unpublished observations). Other important routes of detoxification were through sulfamate and N^2-glucuronide formation. Thus, there is a strong possibility that humans may also transform these compounds in similar fashions. Investigations are underway for detecting some of these polar metabolites of MeIQx that may be present in human urine, in particular, the *N*-hydroxy-*N*-glucuronide, which is an indirect measurement of metabolic activation. The development of such biomarkers may enable us to better evaluate the health risk of chronic dietary consumption of low amounts of heterocyclic amines.

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